ISSN 1330-9862
(FTB-2473)

original scientific paper

Ethanol Production from Different Intermediates of Sugar Beet Processing

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Received: March 11, 2010
Accepted: May 26, 2010

Summary

In this investigation, the production of ethanol from the raw sugar beet juice and raw sugar beet cossettes has been studied. For ethanol production from the raw sugar beet juice, batch and fed-batch cultivation techniques in the stirred tank bioreactor were used, while batch ethanol production from the raw sugar beet cossettes was carried out in horizontal rotating tubular bioreactor (HRTB). In both cases, *Saccharomyces cerevisiae* was used as a production microorganism. During batch ethanol production from the raw sugar beet juice, ethanol yield was 59.89 g/L and production efficiency 78.8 %, and in fed-batch process the yield was 92.78 g/L and efficiency 93.4 %. At the same time, ethanol production in HRTB from the raw sugar beet cossettes with inoculum of 16.7 % *V/m* (raw sugar beet cossettes) resulted in the highest ethanol yield of 54.53 g/L and production efficiency of 79.5 %. The obtained results clearly show that both intermediates of sugar beet processing can be successfully used for ethanol production.

Key words: ethanol, fermentation, raw sugar beet juice, raw sugar beet cossettes, stirred tank bioreactor, horizontal rotating tubular reactor (HRTB)

Introduction

Ethanol is one of the most important biofuels that significantly contribute to the reduction of negative environmental impacts generated by the use of fossil fuels. At the moment, the production costs of biofuels are higher than production costs of gasoline from fossil oil. Therefore, such productions are supported (legislatively or financially) by the governments acting in the direction of replacing fossil fuels by biofuels (*e.g.* USA, Brazil, EU countries) (*1–5*). The development of cost-effective technologies for the production of biofuels is a priority for many research institutions. One of the most promising approaches for the design of cost-effective process configurations is process integration of all operations involved in the ethanol production, which can be achieved through the development of integrated bioprocesses that combine different steps into one single unit. Thus, the integration of reaction and separation steps by removing ethanol from the zone where the biochemical reaction takes place offers several opportunities to increase product yield and consequently to reduce product costs. Process integration is gaining more and more interest due to the advantages related to its use in ethanol production: reduction of energy costs, decrease in the size and number of process units, intensification of the biological and downstream processes (*5–12*).

In the tropical climate, sugar cane is the main source for ethanol production (*e.g.* Brazil) and corn is the dominant raw material in the USA. In the European moderate climate the most convenient renewable raw materials for ethanol production are grains and sugar beet (*13–15*). Due to the surplus of sugar production in the European Union (EU), there is a possibility to redirect the sugar production from sugar beet towards ethanol. In

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terms of the design of ethanol production processes from sucrose-containing materials, the availability and transport costs of the feedstock continue to play a crucial role when new cost-effective production facilities are planned despite the relative maturity of the involved conversion technologies (*5*,*8*,*11*). According to the available technical equipment and current 'state of the art' technology for the fermentative production of ethanol, it is possible to use some intermediate products from sugar production (*e.g.* raw sugar juice with 14–18 % sugar or concentrated sugar syrup with 65–67 % sugar) as well as by-products (*e.g.* molasses containing approx. 50 % sugar). Traditional production of raw sugar juice is characterized by water extraction from sugar beet cossettes. The obtained raw sugar juice can be used either directly for ethanol and sugar production, or it can be concentrated in an evaporator and stored for several months. Raw sugar beet juice and/or concentrate can be used both for sugar production by crystallization and for fermentative ethanol production. For sugar production, raw juice needs to be purified by pulp separation and microfiltration to remove bigger particles, high molecule colourants, proteins and microorganisms. Purified juice (permeate) can be subjected to evaporation and crystallization to obtain sugar. Retentate from microfiltration (all nitrogen components, beet tissue and other impurities) can be mixed with raw sugar beet juice and used for fermentative ethanol production (*16*,*17*).

The aim of this work is to evaluate the ethanol production from different intermediates of sugar beet processing such as raw sugar beet cossettes and juice in order to improve the efficiency of this bioprocess. For ethanol production from raw sugar beet juice, batch and fed-batch cultivation techniques were used during fermentation in stirred tank bioreactor. The newly designed type of horizontal rotating tubular bioreactor (HRTB) was used for ethanol production from raw sugar beet cossettes (semi-solid-state fermentation).

Materials and Methods

Microorganism, media and inoculum preparation

In this research, yeast *Saccharomyces cerevisiae* was used as production microorganism. It was maintained on malt extract agar, which was also used for CFU determination. Raw sugar beet juice and raw sugar beet cossettes (containing approx. 14–16 % of sucrose) were used as media for fermentative ethanol production. For inoculum preparation, raw sugar beet juice was used with the addition of 1 g/L of $NH_4H_2PO_4$ as a source of nitrogen and phosphorus. Media were sterilized at 121 °C for 20 min prior to yeast inoculation. The required amount of yeast suspension for ethanol production in bioreactors (strirred tank bioreactor and HRTB) was obtained by yeast cultivation on rotary shaker at 100 rpm and 28 °C for 72 h.

Ethanol production from raw sugar beet juice in stirred tank bioreactor

Ethanol production from raw sugar beet juice in stirred tank bioreactor (STB) at 28 °C was studied. The bioreactor was inoculated with 10 % (by volume) yeast suspension previously grown on rotary shaker. The working volume of STB was 5 L and the bioreactor was sterilized together with the medium (raw sugar beet juice with 1 g/L of $NH_4H_2PO_4$) at 121 °C for 20 min prior to inoculation. Batch and fed-batch cultivation techniques were used for ethanol production from raw sugar beet juice. During batch process, pH value was maintained in the range from 4.5 to 5.0 by the addition of 0.1 M NaOH and 0.1 M H_2SO_4. Aeration was conducted for the first 12 h of fermentation and pO_2 level was maintained at approx. 30 % of air saturation by the alteration of stirrer speed and air flow rate. Batch phase of the fed-batch process was done in the same way. The feeding in the fed-batch process started when carbon source was almost completely depleted. It was performed by the addition of a few portions of concentrated sugar beet juice (which contained approx. 800 g/L of sugar) depending on the rate of substrate depletion. In this experiment, feeding was done five times by the addition of 200 mL of concentrated sugar beet juice. Fed-batch cultivation was conducted until the constant ethanol concentration was reached.

Ethanol production from raw sugar beet cossettes in horizontal rotating tubular bioreactor

In this investigation, ethanol production from raw sugar beet cossettes in a new type of HRTB was also examined. HRTB is constructed as a 0.60 m long stainless steel tube with an inside diameter of 0.25 m. The interior of the HRTB contains two paddles (0.04 m height and 0.6 m length) fixed on the bioreactor wall in order to improve mixing and homogenization of the bioreactor content. The bioreactor was placed on the bearings that enable rotation of the whole bioreactor. Ethanol production in HRTB was done at room temperature after bioreactor sterilization at 121 °C for 30 min. Batch cultivation technique was used for ethanol production. Fermentation of 5 kg of nonsterile raw sugar beet cossettes (23 % dry matter) started by the addition of different quantities of inoculum (9.1–23.7 % *V/m* of raw sugar beet cossettes) in order to define the broth minimal liquid content. During this research, HRTB was periodically rotated due to the bioreactor content homogenization. The process of ethanol fermentation was monitored by sampling the liquid and the solid part (beet cossettes) of the broth. After that, the cossettes were pressed to obtain liquid samples for analytical purposes.

Analytical methods and bioprocess efficiency parameters

Yeast growth was monitored in the liquid phase of the samples by centrifuging and drying at 105 °C for 48 h. Yeast growth during ethanol production in STB was also monitored by measuring the absorbance at 600 nm. The CFU number was determined by standard microbiological methods (Petri dishes were incubated at 28 °C for 48 h). During fermentation in HTRB, dry mass of sugar beet cossettes was determined gravimetrically. Liquid samples from both fermentations were centrifuged for 15 min at 4500 rpm. Supernatants were used for substrate (sucrose, glucose and fructose), product (ethanol) and by-product (glycerol, acetate and lactate) determina-

tion by HPLC with Supelcogel™ C-610H column (Shimadzu CLASS-VP LC-10A$_{VP}$, Shimadzu, Kyoto, Japan). All fermentation experiments were conducted in duplicate and all samples were analysed in triplicate. Bioprocess efficiency parameters were determined by standard procedures. Ethanol conversion coefficient ($Y_{P/S}$) was calculated by the following equation:

$$Y_{P/S}=\frac{P-P_0}{S_0-S}=\frac{\Delta m_{P,T}}{\Delta m_{S,T}} \qquad /1/$$

where P and P_0 are ethanol concentration at the end and the beginning of fermentation, respectively and S_0 and S are substrate concentration at the beginning and the end of process, respectively. Furthermore, $\Delta m_{P,T}$ is the total mass of the product obtained in the bioreactor, and $\Delta m_{S,T}$ is the total mass of consumed substrate in the bioreactor.

Bioprocess efficiency (E) was estimated as a ratio between experimental ($Y_{P/S}$) and theoretical conversion coefficient ($Y_{P/S})_T$:

$$E=\frac{Y_{P/S}}{(Y_{P/S})_T} \qquad /2/$$

where $(Y_{P/S})_T$=0.538 g/g is theoretical conversion coefficient of sucrose into ethanol.

Bioprocess productivity (Pr) was determined by the following equation:

$$\mathrm{Pr}=\frac{P-P_0}{t}=\frac{\Delta m_{P,T}}{Vt} \qquad /3/$$

where t is cultivation time and V is working volume of the bioreactor.

Results and Discussion

In this research, ethanol production from different intermediates of sugar beet processing (cossettes and juice) was studied. For ethanol production from raw sugar beet juice, batch and fed-batch cultivation techniques were used in STB. The newly designed type of horizontal rotating tubular bioreactor (HRTB) was used for ethanol production during semi-solid-state fermentation on raw sugar beet cossettes.

Ethanol production from raw sugar beet juice in stirred tank bioreactor

A preliminary study was performed in order to define the suitability of raw sugar beet juice as a medium for ethanol production. These preliminary results (data not shown) indicated that it is necessary to correct the content of raw sugar beet juice in order to achieve adequate yeast growth. For that purpose, $NH_4H_2PO_4$ was added as a source of nitrogen and phosphorus. From shake flask research (data not shown), it was concluded that the addition of 1 g/L of $NH_4H_2PO_4$ to raw sugar beet juice is sufficient for considerable increase of yeast growth and ethanol production. It was also observed that 12-hour aeration at the beginning of fermentation considerably increased yeast growth and accelerated ethanol production.

Batch process of ethanol production from raw sugar beet juice with 1 g/L of $NH_4H_2PO_4$ is presented in Figs. 1 and 2. After sterilization and cooling at the working temperature of 28 °C, bioreactor was inoculated with 10 % (by volume) yeast suspension previously grown on rotary shaker. As it can be seen in Fig. 1, sugar was completely consumed after 78 h whereby ethanol, as the main fermentation product, and glycerol, as the main by-product, were obtained. At the end of the fermentation, total ethanol concentration was 63.12 g/L and glycerol concentration 9.7 g/L. However, the net production of ethanol in this fermenation was 59.89 g/L due to the fact that 3.16 g/L of ethanol was introduced by inoculation. In this batch process, yeast growth was observed only in the first 12 h of fermentation as a consequence of the avialability of oxygen, nitrogen and phosphorus sources in the broth (Fig. 2). After that period, biomass concentration (X) and yeast cell number (log N) were at approximately constant level due to the fact that yeast started

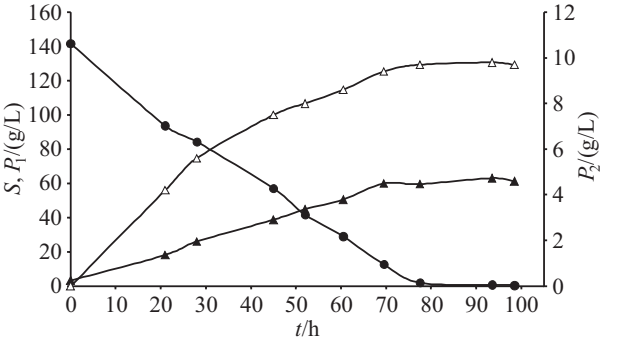

Fig. 1. Alteration of substrate (S, ●), ethanol (P_1, ▲) and glycerol (P_2, Δ) concentration during batch fermentation of raw sugar beet juice in stirred tank bioreactor

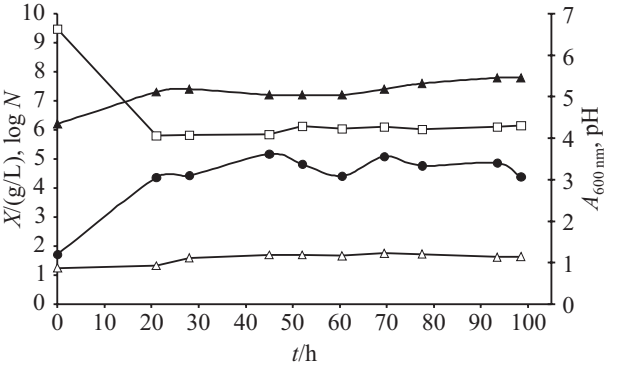

Fig 2. Alteration of dry biomass (X, ●), viable cell number (log N, ▲), broth absorbance ($A_{600\ nm}$, Δ) and pH (□) during batch fermentation of raw sugar beet juice in stirred tank bioreactor

to produce ethanol under anaerobic conditions. As it can be seen in Fig. 2, changes of absorbance (A) were in agreement with the changes of biomass concentration (X) and viable cell number (log N). Therefore, the broth absorbance can be used as an indicator of biomass growth. Changes of pH value during the fermentation are in agreement with the rate of substrate consumption. The productivity of batch process was 0.608 g/(L·h).

A fed-batch process was also investigated in order to increase the efficiency of ethanol production. Initial substrate concentration in the medium was reduced to 75 g/L to avoid substrate inhibition effect at the beginning of fermentation. During fed-batch process, feeding was performed by the addition of a few portions of concentrated raw sugar beet juice (containing approx. 800 g/L of sugar). Dynamics of feeding process was controlled depending on the substrate consumption rate and the new feed portion was added to the bioreactor when substrate concentration dropped below 10 g/L. Batch phase was carried out under the same conditions as described in the previous experiment. As it can be seen in Fig. 3, permanent increase of ethanol concentration was observed during feeding, which is an indicator that feeding dynamics is in agreement with ethanol production by yeast cells. At the end of this process, ethanol concentration was 97.99 g/L, which is in agreement with literature (*16*,*17*). At the same time, constant increase of glycerol concentration was also detected during feeding, which can be explained as a consequence of substrate availability under these conditions. Glycerol concentration at the end of fed-batch process was 9.84 g/L, which is in agreement with previous results. Again, as observed in the batch process, absorbance was a good indicator of yeast growth. In fed-batch process, higher ethanol production efficiency (E=93.4 %), but lower productivity (Pr= 0.503 g/(L·h)) were detected compared to the batch process.

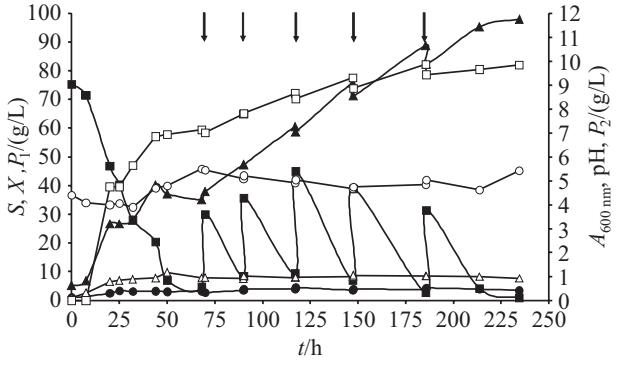

Fig. 3. Alteration of substrate (S, ■), ethanol (P_1, ▲), glycerol (P_2, □) and biomass (X, ●) concentration, pH (○) and broth absorbance ($A_{600\ nm}$, Δ) during fed-batch fermentation of raw sugar beet juice in stirred tank bioreactor. Arrows represent the addition of concentrated fresh medium during the fed-batch process

Ethanol production from raw sugar beet cossettes in HRTB

During this research, ethanol fermentation was monitored by sampling both liquid and solid part (beet cossettes) of the broth. After the fermentation, the cossettes were pressed to obtain liquid samples for analysis. The main goal of this investigation was to define the minimal liquid content of the broth that is required for ethanol fermentation with yeast *S. cerevisiae*. Therefore, ethanol fermentation in HTRB was performed with different initial volumes of inoculum (9.1–23.7 % V/m of raw sugar beet cossettes) at room temperature ((20±2) °C). On the basis of preliminary fermentations (data not shown), it was proven that raw sugar beet cossettes have sufficient amount of nitrogen and phosphorous sources for yeast growth so it was not essential to add inorganic salt to the broth. During these fermentations, HRTB was periodically rotated in order to homogenize the bioreactor content. At the beginning of all experiments in HRTB, dry matter content of raw sugar beet cossettes was 23 % and at the end of experiments it was 12 %. Sugar concentration in the juice squeezed from raw sugar beet cossettes was approx. 110 g/L. In all experiments, it was observed that sugar concentration in the liquid part was lower than in the solid part of the broth due to the fact that certain time (approx. 20 h) is required for sugar diffusion from the cossettes into the liquid. Furthermore, at the start of all HRTB experiments ethanol concentration was approx. 15 g/L as a consequence of ethanol addition with the inoculum. Initial yeast cell number in the liquid part of the broth was approx. 10^8 CFU/mL. It was noticed that sampling procedure could be a reason for errors in the fermentation monitoring due to the heterogeneity of the solid part of the broth. In order to obtain an accurate view of ethanol fermentation under these conditions, it was necessary to take samples from the liquid as well as from the solid part of the fermentation broth. As an example of ethanol fermentation from raw sugar beet cossettes in HRTB, fermentation with the inoculum of 16.7 % V/m (raw sugar beet cossettes) was selected. Results of this fermentation are presented in Figs. 4–6. As it can be seen in Figs. 4 and 5, the substrate was almost completely depleted after 50 h, in both liquid and solid part of the fermentation broth. This phenomenon is similar to the sugar beet juice fermentation in stirred tank bioreactor and it can be explained by the fact that liquid content in the broth during fermentation with inoculum of 16.7 % V/m (raw sugar beet cossettes) is sufficient for successful ethanol fermentation in HRTB. Due to the substrate consumption from the fermentation broth, dry matter of the cossettes was reduced for the quantity of consumed sugar. Ethanol and by-products (glycerol and acetate) were synthesized as a consequence of substrate consumption. At the end of fermentation, ethanol concentration in the solid part (beet cossettes) was 67.89 g/L (Fig. 4) and in

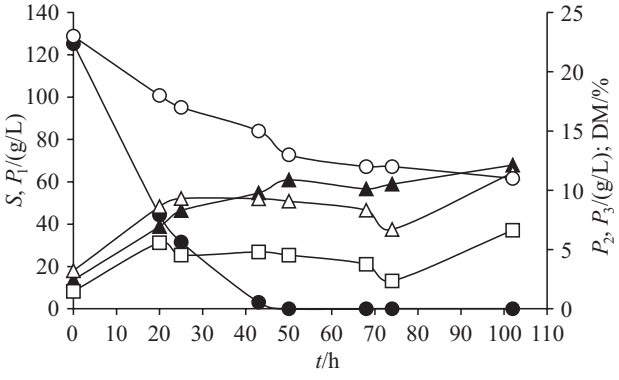

Fig. 4. Alteration of substrate (S, ●), ethanol (P_1, ▲), glycerol (P_2, Δ) and acetate (P_3, □) concentration and dry mass of sugar beet cossettes (DM, ○) during fermentation in HRTB with the inoculum of 16.7 % (V/m)

the liquid part of the broth was 62.9 g/L (Fig. 5). On the basis of these results, it is clear that raw sugar beet cossettes can be used directly for ethanol fermentation, but more detectable by-products (*e.g.* glycerol, acetate and lactate) were produced compared to the fermentation on raw sugar beet juice. This can be explained by

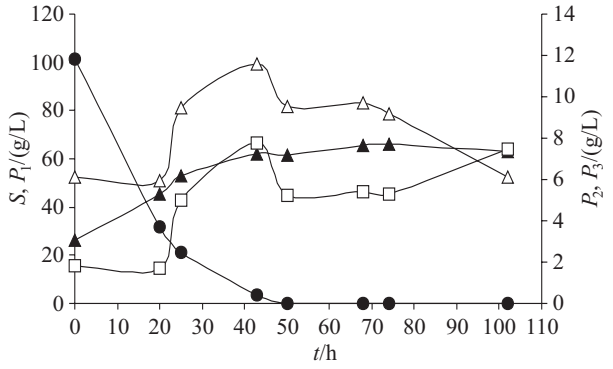

Fig. 5. Alteration of substrate (S, ●), ethanol (P_1, ▲), glycerol (P_2, Δ) and acetate (P_3, □) concentration in the liquid part of the fermentation broth in HRTB with the inoculum of 16.7 % (V/m)

the presence of other microorganisms on nonsterile raw sugar beet cossettes. At the end of this experiment, the by-product (glycerol and acetate) concentrations were in the range from 6.1 to 11.5 g/L. As it can be seen in Fig. 6, pH value was reduced in the liquid part of the medium as a consequence of substrate consumption and by--product synthesis (*e.g.* acetate and lactate). The expected slight increase of dry biomass and cell number were observed due to the biomass growth. Some oscillations in the dry biomass were detected as a consequence of experimental errors and system heterogeneity. Temperature was kept at approximately constant level during the whole experiment, which indicates that the fermentation process was carried out at approximately constant rate (Fig. 6). In all other experiments of ethanol production from raw sugar beet cossettes in HRTB similar fermentation results were obtained.

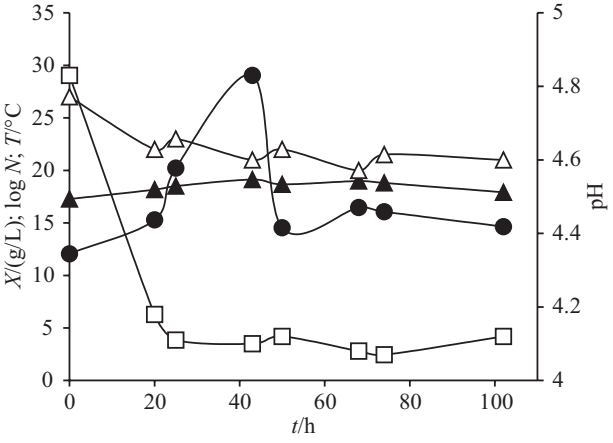

Fig. 6. Alteration of dry biomass (X, ●), viable cell number (log N, ▲), pH value (□) and temperature (T, Δ) in the liquid part of the fermentation broth in HRTB with the inoculum of 16.7 % (V/m)

Comparison between different systems for ethanol production

Comparison between different systems for ethanol production was done on the basis of bioprocess efficiency parameters such as conversion coefficient ($Y_{P/S}$), efficiency (E) and productivity (Pr). As it can be seen in Table 1, the obtained ethanol concentration in batch fermentation using the raw sugar beet juice was 59.89 g/L and productivity 0.608 g/(L·h). The efficiency of the batch fermentation of raw sugar beet juice was 78.8 %. It is defined as a ratio between experimental and theoretical conversion coefficients. For theoretical conversion coefficient, sucrose conversion coefficient was chosen due to the fact that sucrose is a prime sugar (>95 % of total sugar content) in sugar beet juice (*17*). During fed-batch process, the highest ethanol concentration (97.99 g/L) was observed. Therefore, the highest conversion coefficient (0.502 g/g) and efficiency (93.4 %) were also detected. However, due to the relatively long time period (234 h) required to reach this ethanol concentration, productivity was lower than in batch process (0.503 g/(L·h)). During the study of ethanol production from the raw sugar beet cossettes in HRTB with different inoculum quantities (9.1–23.1 % V/m raw sugar beet cossettes), total mass of ethanol in the bioreactor was in the range from 158.87 to 242.90 g with bioprocess productivity in the range from 0.357 to 0.497 g/(L·h) (Table 1). Furthermore, the efficiency of ethanol production was in the range from 52.3 to 79.5 %. It is clear from the obtained results that the highest total mass of ethanol, conversion coefficient and efficiency were detected in the experiment with inoculum of 16.7 % V/m (raw sugar beet cossettes) in HRTB. Further increase of the inoculum volume had as a consequence an increase of ethanol concentration in the liquid part of fermentation broth in HRTB. This phenomenon can be explained by the fact that the increase of free water content in the broth is related to the more effective diffusion of sugar and ethanol from the cossettes. Under these conditions, further increase of ethanol production efficiency parameters was not detected and therefore, it can be concluded that the inoculum of 16.7 % (V/m) is sufficient for fermentation process. Increase of inoculum quantity in HRTB is also related to the increase of free water content in the broth, which consequently has an impact on the energy demand for ethanol separation by distillation. Comparison between ethanol production from raw sugar beet cossettes and juice pointed out slightly higher bioprocess efficiency during fermentation of raw sugar beet juice. This result clearly shows that raw sugar beet cossettes can be successfully used for ethanol production, which could simplify it considerably from the point of view of energy and economy.

Conclusions

On the basis of the obtained results, it is clear that both intermediates of sugar beet processing can be successfully used for ethanol production. Raw sugar beet juice after the addition of nitrogen and phosphorus sources is a suitable complex medium for ethanol production. The use of appropriate cultivation techniques (fed--batch or repeated batch) can significantly increase etha-

Table 1. Comparison among different systems for ethanol production from intermediates of sugar beet processing

Production system	t/h	$m_{\Delta S,T}$/g	$m_{\Delta P,T}$/g	$Y_{P/S}$/(g/g)	E/%	Pr/(g/(L·h))
RSBJ+STB+BP	98.5	706.50	299.45	0.424	78.8	0.608
RSBJ+STB+FBP	234	1170.00	587.96	0.502	93.4	0.503
RSBC+HRTB (9.1 % V/m of INM)	93	599.54	231.25	0.386	71.7	0.497
RSBC+HRTB (13 % V/m of INM)	99	622.00	229.60	0.369	68.6	0.464
RSBC+HRTB (16.7 % V/m of INM)	102	566.90	242.90	0.428	79.5	0.476
RSBC+HRTB (20 % V/m of INM)	90	571.20	160.63	0.281	52.3	0.357
RSBC+HRTB (23.1 % V/m of INM)	68	516.20	158.87	0.308	57.3	0.467

RSBJ – raw sugar beet juice, RSBC – raw sugar beet cossettes, STB – stirred tank bioreactor, BP – batch process, FBP – fed-batch process, INM – inoculum

nol yield and consequently improve the performance and economics of the process. Ethanol yield in these experiments was in the range from 59.89 to 92.78 g/L and efficiency in the range from 78.8 to 93.4 %. The use of raw sugar beet cossettes in ethanol production eliminates the extraction of sugar beet cossettes by hot water, which considerably reduces energy demand for ethanol production. At the same time, this fact also has a considerable impact on the final price of ethanol as a fuel. Furthermore, it is necessary to point out that further research of ethanol production from the raw sugar beet cossettes is required, combined with the improvement of sampling techniques due to the system heterogeneity in HRTB.

Acknowledgement

The authors are grateful for financial support provided by the Ministry of Science, Education and Sports of the Republic of Croatia (Grant no. 058-0581990-2004).

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